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Protein and Amino Acid Content of Uterine and Oviductal Fluid of Dairy Heifers.

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PROTEIN AND AMINO ACID CONTENT OF UTERINE AND OVIDUCTAL FLUID OF DAIRY HEIFERS

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ABSTRACT

Thirty nulliparous dairy heifers of breeding size (275-325 kg) and age (16-18 months) were used to study the effects of 4 different management systems on protein and amino acid content of uterine and oviductal fluids. Groups were identified according to the type of management the dairy heifers received: Group I, non-synchronized estrus and under natural summer conditions of Louisiana; Group II, synchronized estrus and under natural summer conditions of Louisiana; Group III, second estrus after withdrawal of MGA and maintained in an environmental control chamber for one estrous cycle under hot conditions (simulating conditions of Groups I and II); Group IV, second estrus after withdrawal of MGA and maintained under cool conditions of late fall and early winter of Louisiana. Animals in Groups III and IV were slaughtered at the termination of the experiment and uterine fluid and physiological saline flushings of the ampulla and the isthmus collected after exsanguination. Uterine fluid was collected on day 0 (8-12 hours after onset of standing estrus) and day 2 (48 hours after day 0).

Protein levels in uterine fluid varied among animals and treatments. The average protein level varied from a low of 0.43 mg/ml for day 2 of Group III to a high of 1.68 mg/ml for day 2 of Group II. Ampullary and isthmic flushings from Groups III and IV contained on the average 0.20-0.21 mg/ml and 0.16-0.17 mg/ml, respectively.

Amino acids in uterine fluid of Group IV in descending order of occurrence were, glutamic acid, threonine, alanine, glycine, arginine (in 10 out of 10 samples), serine (8/10), lysine (5/10), leucine (3/10) and valine (1/10). These amino acids were also found in uterine fluid from Groups I, II and III. Additional amino acids detected in other groups were, unknowns 1 and 2, (Groups I, II, III), taurine (Groups II, III), aspartic acid (Group I), phenylalanine and histidine (Groups I, II), and ornithine (Group II). The major amino acids detected in uterine fluid were also observed in physiological saline flushings of the ampulla and the isthmus. Amino acid levels were less in these flushings due, at least in part, to dilution.

CHAPTER I

INTRODUCTION

Thirty to 40% of all mammalian embryos die before birth, and most die within the first week after conception (12). Bishop (6) suggests that a part of this loss is unavoidable and should be regarded as a normal way of eliminating unfit genotypes in each generation. The other part of the loss is probably caused by a variety of factors and is of concern to many reproductive physiologists.

The term "uterine environment" is familiar to all of those closely associated with reproductive physiology and to many not intimately associated with this area. This term is used to describe the overall characteristic of the internal or luminal area of the female reproductive tract and is used loosely to include not only the lumen of the uterus but also the uterine horns as well as the isthmus and the ampulla or oviduct. Although the term "uterine environment" is in rather common usage, its exact physiological and biochemical meaning is not well understood. The role or importance of the uterine environment in embryonic mortality is not well documented.

Embryonic death is one of the major factors involved in low reproductive efficiency and it is generally agreed that most of the embryonic losses occur during the first few days after conception. The cause or causes of these losses are not known, but it appears

that the makeup of the uterine environment and/or uterine fluid is important if fertilization is to take place and if most embryos are to survive this critical period.

Spermatozoa traverse the uterus, isthmus and into the ampullary region where fertilization occurs and are, therefore, exposed to the uterine environment. Zygotes obtain nourishment from the uterine fluid, a part of the uterine environment, until attachment or implantation occurs. The fetus must survive in the uterus throughout gestation. The nutrient requirements of developing blastocysts are being studied both in vivo and in vitro, but these requirements are not well established for any species of animals. There appears to be considerable variation between species on nutrient requirements for embryo development. Therefore, research must be conducted with domestic animals, in addition to the common laboratory animals, if improvements in reproductive efficiency are to be achieved.

All factors affecting uterine environment are probably not known, but some of the factors that have been suggested as being involved with reproductive efficiency are heat stress, hormones, intra-uterine devices, nutrition and diseases. The relationship of these with uterine environment and reproductive efficiency is being studied to determine whether any or all can help slow the human population explosion and also to try to find ways of improving reproductive efficiency in domestic animals.

A better understanding of the uterine environment should make it possible to develop suitable methods of controlling the

human population explosion and of increasing the number of embryos in domestic animals that survive to produce milk and meat. When the physiological and biochemical properties of the uterine environment are better understood, perhaps the problems now associated with low reproductive efficiency, such as "hard breeders," low conception rate associated with estrus synchronization, and embryonic death, will be better understood.

Research reported in this presentation was planned and conducted to evaluate the uterine environment of dairy heifers under various systems of management. The primary objectives of this research were: (1) to determine the level of protein and the number and amount of amino acids in uterine fluid of non-synchronized dairy heifers; (2) to determine the effect of feeding Melengestrol acetate (MGA) for estrus synchronization on the composition of uterine fluid; and (3) to study the effect of heat stress on the composition of uterine fluid of dairy heifers.

CHAPTER II

REVIEW OF LITERATURE

A. Uterine Environment

The term uterine environment, as introduced previously, is inclusive of the overall characteristics of the uterus and oviduct lumen as well as the fluid in these organs. This fluid has been called uterine milk by several writers (3,4,23,32,34,74) in an apparent effort to indicate that developing zygotes obtain part or all of their nourishment from this fluid. The term uterine milk was first used by Needham in 1667, according to Amoroso (1), and this writer further states that studies on the chemical composition of uterine milk were made in 1842, 1855, and 1864. Some of the properties studied and the quantity of each were: dry matter 12.0%; ash 0.5%; protein 10.0%; fat 1.0%; and no glucose. In the last half of the nineteenth century it was established that spermatozoa entered into the ovum at fertilization (9). After this important discovery most investigations centered around fertilization and cleavage. The chemical composition of uterine fluid and/or the significance of this fluid was not studied extensively for several years. Bishop (5) in a recent textbook (1969) indicated that even to date surprisingly few species have been investigated as to the chemical composition of the contents of the oviduct and "none have been explored with thoroughness."

Little interest was shown in the chemical composition of uterine and oviductal fluids for several years for two basic reasons: (1) a satisfactory method of collecting the fluid from live animals was not available and (2) only a relatively small volume of fluid was available from this organ. Development of refined biochemical techniques made it possible to analyze uterine and oviductal fluid, but a method or technique for collecting uterine and oviductal fluid from live animals was still needed.

B. Collection Techniques

It is difficult to identify the point in recent history when a renewed interest in the chemical composition of uterine and oviductal fluid or secretions was revived. Certainly one of the significant contributions was made by Bishop (3) when he successfully cannulated the oviduct of a rabbit and determined the rate of secretion of oviductal fluid. It was also demonstrated that both secretory rate (avg. vol. 0.79 ml/24 hours/tube) and pressure in a ligated oviduct were highest during estrus and lowest in late pregnancy. The secretory activity of the rabbit oviduct was greatly reduced after castration but the secretory activity could be restored with the administration of estradiol benzoate.

Techniques used to obtain uterine fluid in early research were varied and included the following: (1) laparotomy and ligation in rabbits (3); (2) putting reproductive tracts of slaughterhouse cattle through a hand operated clothes wringer (51); and (3) a washing or flushing of the reproductive tracts of live animals (32). In 1960 Clewe and Mastroianni (13,14) were successful in developing a technique

for continuous collection of oviductal fluid from rabbits. This method of collecting oviductal fluid was used successfully by other workers (34,53) and was later adapted for collecting fluid from ewes (8,53,55) and sows (18). Fahning et al. (20) in 1966 described a new and different technique for collecting uterine fluid from cows by aspiration and concluded that collection of uterine fluid by this means did not significantly lower future reproductive efficiency of these cows.

Development of a continuous collection technique for rabbits, ewes, and sows made it possible to study the cyclic nature of secretions from the oviduct. Lombard et al. (43) noted in earlier work with virgin heifers that a mucous-like material was more abundant in the bovine oviduct at 3 to 4 days postestrus than in the other periods of the estrous cycle. The known association of estrogen and progesterone with estrus and with the luteal phase of the estrous cycle, and the larger volume of fluid associated with estrus (3,32,51), soon led to studies to evaluate the effects of these hormones on uterine and oviductal secretion.

C. Factors Affecting Uterine Fluid

1. Hormonal

Bishop (3) gave evidence that the active secretion of the oviduct was influenced by ovarian hormones and that estradiol would stimulate secretion of fluid under the conditions of his experiment. Other workers have demonstrated a hormonal influence on the oviductal secretion of several species: women (23), cattle (32,51), rabbits (28,45), ewes (33,48,52,56,64), rats (41), and monkeys (44). Results

of several other studies indicate that estrogenic hormones, particularly estradiol benzoate, stimulate secretion of fluid from the oviduct (45,48,56). Progesterone with estrogen decreased the secretion rate (29,45,48), progesterone alone had no effect (47,56), and progesterone before estrogen shortened the effects of estrogen by 2 to 4 days (56). In ovariectomized rabbits progesterone decreased the secretion rate of the oviduct to less than 20% of that of estrogen treated rabbits (29).

Administration of progestins for estrus synchronization of cattle and sheep can be successful, but there is a lower conception rate in those animals bred the first estrus after withdrawal. A full explanation of this lower conception rate is not available. However, the demonstrated effect of progesterone on oviductal secretion was responsible for stimulating interest in attempting to determine the effect of exogenous hormones on the uterine and oviductal environment (33,37,52,68). In a pilot study (42) with rabbits it was found that a single injection of progesterone caused a decrease in the amino acid content of uterine fluid which was collected 24 hours after administration of progesterone.

2. Heat Stress

Embryonic loss in mammals the first 6 days after conception is high in areas with high environmental temperatures, according to Wiersma and Stott (76), and this embryonic loss is associated with heat stress. It was proposed by these investigators that the higher progesterone levels in the perpherial blood stream of heat stress cattle was due to production of progesterone by the adrenal cortex.

The adrenal gland is one of the endocrine glands that responds to stress situations in the body. It is possible that excessive heat could be sufficient stress to stimulate secretion from this gland. It was demonstrated that a 24 hour heat stress would result in an elevated level of progesterone in the perpherial blood. This higher level of progesterone was considered to be partially responsible for the early embryonic loss in animals that are under heat stress. In apparent opposition to this thesis, Thwaites (72), working with sheep 15 days postmating, states that his results tend to exclude endocrine function of the corpus luteum, thyroid, and adrenal cortex as mechanisms of embryo mortality in heat stress ewes. He concludes, however, that the changes in the luminal fluid of the Fallopian tubes and uterus are the most likely mechanism of heat-induced embryo mortality. Howarth and Hawk (35) observed that hydrocortisone acetate (HCA) had no effect on fertilization in ewes but did significantly reduce embryonic survival in two experiments conducted during late summer and early autumn. However, HCA had virtually no effect on fertility in experiments conducted during the winter months.

3. Intrauterine Devices

The effects of intrauterine devices (IUD) on the uterine environment is of interest in the overall evaluation of the composition of uterine fluid. The exact mechanism whereby IUD's prevent conception, implantation and/or cause embryonic death is not known. Several species of animals have been studied with respect to IUD's and there may be a species difference in response to the presence of IUD's in the reproductive tract. It is interesting to note that DeBoer and

associates (16) proposed that it was possible that a substance produced in the IUD horn of rats was migrating into the non-IUD horn and preventing implantation and therefore causing embryonic loss. The chemical nature of this material was not determined.

Brinsfield and Hawk (10,31), working with ewes, have demonstrated that IUD's affect motility of the uterus and that this is important in sperm transport or lack of transport. Also, these workers reported that IUD's intensify the spermicidal and bactericidal properties of the uterus or uterine environment. Cooper and Hawk (15) reported that IUD's promoted uterine growth, thus simulating the effects of estrogen. Dukelow and associates (17) demonstrated a reduction in the protein content of uterine and oviductal secretions of rabbits when IUD's were present in the reproductive tracts. Uterine fluid decreased in protein content from 6.7 mg/ml to 1.3 mg/ml and the oviductal fluid from 5.9 mg/ml to 2,7 mg/ml. The number of amino acids in uterine and oviductal fluids was less in IUD treated animals.

The cyclic nature of fluid secretion in the oviduct and uterus due to hormonal influence, the effects of heat stress on the level of progesterone in perpherial blood, lower conception rate associated with feeding progestins for estrus synchronization and the effect of IUD's are all closely related to and directly associated with uterine environment. It is possible that part of the effects of the above are simply the effects each exerts individually or collectively on the chemical composition of the uterine and oviductal fluids.

D. Composition of Uterine and Oviductal Fluid

1. Rabbit

Much of the early work on chemical composition of uterine and oviductal fluids was done with rabbits. Oviductal secretions were studied more extensively after the successful cannulation of the oviduct (3) and a continuous collection technique (13) developed. Mastroianni and Wallach (46), using paper chromotography found that the protein and peptide concentration in oviductal fluid were not altered following mating. Glycine, glutamic acid, alanine, valine and leucine were consistently present in oviductal fluid, and cysteic acid, serine, taurine and glutamine were detected in some of the samples. Gregoire et al. (22) identified the free amino acids in the tubal and uterine fluid of intact, castrated, estrogen and progesterone treated rabbits. The major amino acids isolated were glycine, serine, glutamic acid, alanine and threonine. Progesterone increased the glycine and serine content eight-fold in the uterine fluid and two-fold in the tubal fluid. The reverse was found to be true in 3 out of 4 rabbits injected with a single dose of progesterone in a 2X2 latin square design experiment (42), that is, progesterone decreased the concentration of the four major amino acids found in the uterine fluid of rabbits. Engle et al. (19) detected 18 amino acids in the oviductal fluid of rabbits and sows with the major amino acid being glycine. Threonine and serine were found to be significantly ($P < .05$) higher in the oviductal fluid of rabbits than in that of the sow. The level of amino acids in the oviductal fluid of sows ranged

from 0.31 mg/100 ml for methionine to 20.74 mg/100 ml for glycine, and oviductal fluid of does ranged from 0.18 mg/100 ml for tyrosine to 3.90 mg/100 ml for glycine.

Using moving boundary electrophoresis, Stevens et al. (71) observed eight electrophoretic components in the uterine fluid collected from ligated rabbit uteri. At least two of these classes of proteins were not present in the blood sera of these rabbits and one of the proteins migrated as a pre-albumin and the other as an alpha-globulin. Kirshnan and Daniel (40) have isolated a protein they called "blastokinin" and Hamana and Hafez (27) a protein called "uteroglobin" that induces blastulation. This protein, assuming them to be the same (27), is found only in the rabbit uterus from day 3 to day 9 of gestation.

2. Sheep

An apparatus for the continuous collection of fluid from the oviduct of sheep by cannulation was described by Black et al. (8). Other workers (55,57,64,65) have used various modifications of the same technique and the technique was further modified by Perkins et al. (53) to collect fluid from the oviduct and uterus in separate containers. This made it possible to study the relationship of the two fluids collected simultaneously from one animal.

Rowan (64) in 1965 reported the average concentration of nitrogen in oviductal fluid of ewes to be 613 mg/100 ml with a range for the estrous cycle of 477 mg/100 ml to 720 mg/100 ml. A higher concentration of total nitrogen was found 5 to 6 days after estrus and the lowest amount 7 days before estrus. The average for the day

of estrus was 640 mg/100 ml. Rowan and Goode (65) reported that nitrogen concentration did not vary significantly during the estrous cycle and that peak flow was reached 2 days after the onset of estrus.

Restall and Wales (57) reported that uterine fluid from normal ewes contained 1.36 g of protein/100 ml and that the protein content increased to 2.90 g/100 ml in spayed ewes but the volume of fluid decreased. The cyclic nature of fluid flow or secretion by the oviduct in the ewe is well documented (7,33,36,48,52,56,57); however, the cause of this phenomenon is still under investigation. Administration of the exogenous hormones, progesterone-pregnant mare serum (P-PMS), for estrus synchronization were found to cause a 24 hour delay in peak flow of fluid from the oviduct of ewes (52). Peak flow was observed on day 3 for the P-PMS treated ewes instead of day 2 as was observed in the untreated ewes (41,65). The average concentration of protein in oviductal fluid was 3.02 g/100 ml. The mean protein content of the fluid had a range of 1.56 to 4.39 g/100 ml but the ewes that had the higher amounts in natural estrus were also found to have higher amounts when in the estrus synchronization group.

Average protein content of uterine and oviductal fluids as reported by Iritani et al. (36) were as follows: oviductal fluid on day 0 to 4 of the estrous cycle, 3.0 g/100 ml; for day 5 to 16, 2.4 g/100 ml; for uterine fluid on day 0 to 4, 3.5 g/100 ml; and day 5 to 16, 4.5 g/100 ml. The cyclic production of fluid was noted in both uterine and oviductal fluid secretion but the total percentage drop in fluid flow was less in the oviduct. The range in oviductal

fluid flow was from a peak of 1.35 ml/24 hours to a low of 0.45 ml/24 hours while the uterine secretion reached a peak flow of 3.43 ml/hour and decreased to a low of 0.59 ml/hour. Perkins et al. (53) reported that the volume of fluid from the uterine horns exceeded that from the oviduct during estrus, but that the oviduct produced a larger volume of fluid than the uterine horn during the luteal phase of the estrous cycle. Restall (54) stated that the secretory cells of the ampulla continually secrete fluid while the isthmus contains very few secretory cells and therefore produces very little fluid at any time.

The least squares means of protein concentration for individual ewes ranged from 0.94 to 2.85 g/100 ml of oviductal fluid (50). Murray et al. (50) observed that the protein content of oviductal fluid did not vary significantly between seasons of the year, among days of the estrous cycle, or between ligated and non-ligated oviducts. Mating did not effect protein concentration in fluid from the non-ligated oviduct. Three out of five ewes with one oviduct cannulated became pregnant, indicating that the cannulation process and collection of fluid from one oviduct did not affect the contralateral oviduct as far as fertilization was concerned.

A total of 17 amino acids in oviductal fluid from ewes was reported by Higgins and Perkins (33). These ewes were allowed one natural cycle following cannulation and then three were estrus regulated with daily injections of 10 mg of progesterone for 14 consecutive days followed by 250 IU of pregnant mare serum. Three other ewes were allowed to continue natural cycling but were given 300 mg of HCA for 4 consecutive days beginning the first day of the second estrus after

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cannulation. Seven amino acids present in all samples were glutamic acid, glycine, valine, leucine, lysine, histidine and arginine. The samples from the naturally cycling ewes contained phenylalanine, in addition to the seven amino acids listed above, and fluid from the estrus regulated group of ewes contained methionine, in addition to those amino acids found in the naturally cycling ewes. Concentration of most free amino acids was lowest in those samples collected during estrus and highest during the luteal phase. Progesterone treatment was associated with increasing amino acid concentration and the protein content of oviductal fluids did not vary significantly with exogenous hormone treatment or with the stage of the estrous cycle, according to these workers (16).

In a study of the ovine cervico-vaginal mucus by Woodward and Sorenson (77) 15 amino acids were identified and quantitated. The mucus from control animals contained the highest concentration of amino acids and varied from a low of 3.7 mcg/g for tyrosine to a high of 28.48 mcg/g for glutamic acid. Although the concentration of all amino acids decreased, the percentage composition for glycine, glutamic acid, lysine and arginine increased considerably toward the end of the estrous cycle.

3. Cattle

One of the earlier attempts to determine the chemical composition of luminal fluid from the bovine female reproductive tract was by Olds and Van Demark (51). These workers removed the reproductive tract from 19 cull dairy cows at slaughter and from 39 slaughterhouse

cattle and collected fluid from the oviduct and uterine horns. The volume of fluid collected from the uterus and uterine horns averaged 0.75 ml with a range of 0 to 8.0 ml. The uterine fluid contained 0.74 g of total nitrogen per 100 ml of uterine fluid. An average of 0.145 g of oviductal fluid was collected from each oviduct and contained 1.95 g of total nitrogen per 100 ml of fluid; however, this average was from one determination of a composite sample from three cows. Little cyclic variation in oviductal fluid content was noted, but a definite variation between individuals was observed.

In 1962 Heap (32), using a flushing technique, reported that flushings from the reproductive tracts of cows contained 80 mcg of total nitrogen per ml of flushing during estrus and 332 mcg/ml of flushing during the luteal phase. The protein nitrogen content was determined to be 64 mcg/ml and 155 mcg/ml for estrus and the luteal phase, respectively. Comparing these results with rabbits, rats and sheep, he found a significant difference between species in relative amounts of the chemical constituents, as well as between the two stages of the cycle.

In 1967 Fahning et al. (21), using a Spinco automatic amino acid analyzer, found a total of 25 amino compounds in uterine fluid and only 23 amino compounds were identified in blood serum. Three amino compounds found in uterine fluid but not in blood serum were ethanolamine, Beta alanine and cystine. Citrulline was found in the blood serum but not in uterine fluid. The concentration of free amino acids was greater in uterine fluid than in blood sera, thus indicating active secretion.

According to Hackett and Hafs (26) the protein synthetic activity per uterine cell (RNA/DNA) nearly doubled during the early luteal phase of the cycle between day 2 and day 7 and then declined almost continuously to day 2 of the following cycle. Five Holstein heifers were slaughtered on each of the following days of the estrous cycle: day 0,4,7,11,18, and 20. The reproductive tracts were removed within 30 minutes after exsanguination. A 5 gram sample of each tract homogenized in distilled water, adjusted to contain 50 mg of tissue per ml, and analyzed for RNA/DNA and protein. The average concentration of protein in this material on the various days of the estrous cycle were: day 0, 18.8 g; day 2, 20.6 g; day 4, 18.5 g; day 7, 25.3 g; day 11, 19.9 g; day 18, 22.8 g; and day 20, 23.4 g. Epithelial cell height of the uterus of these heifers had two peaks during the estrous cycle, on day 0 and day 7.

Histological changes in the cow's oviduct and uterus, following the feeding of progestogens for estrus synchronization, was investigated by Smallwood and Sorenson (68). A highly significant difference was detected in the epithelial cell height of the oviduct of 80 Hereford feedlot heifers 2-5 days after withdrawal of the progestogens. Maximum epithelial cell height was observed on day 4 after withdrawal and this was the day most of the heifers ovulated. It was concluded that this was the expected pattern. Also the vascularity of the uterus was observed and those uteri appearing congested with blood beneath the surface, having petechial hemorrhage or clots of blood in the lumen were classified as vascular uteri. The following results were

noted on days after withdrawal of progestogens: day 2, 19 out of 20 uteri were vascular; day 3, 20 out of 20 were vascular; day 4, 4 out of 20 were vascular; and on day 5, none of the uteri were vascular. Olds and Van Demark (51) in earlier work noted that shortly after estrus the mucus of the reproductive tract was frequently bloody and slightly yellow with a tendency to clot.

In a unique investigation Schultz et al. (66) used three groups of ovariectomized Holstein cows to study the nuclear size of the cells lining the glands of the bovine endometrium. The groups received the following treatment: Group I, 100 mg of progesterone in corn oil; Group II, 3 mg of estradiol-17-Beta in corn oil; and the controls Group III, 5 ml of corn oil. A highly significant difference ($P < .01$), in nuclear size between groups, was detected. The difference was attributed to the influence of steroid hormones on the nuclei of the epithelial cells lining the mucous glands of the endometrium. The size of the nuclei in non-specific units were as follows: Group III, 29.19; Group II, 31.19; and Group I, 33.58. The nuclei in Group III (control) were smaller than the other groups, Group I (progesterone treated) had the larger nuclei, and Group II was intermediate.

CHAPTER III

EXPERIMENTAL MATERIALS AND METHODS

A. General Procedure

Thirty nulliparous dairy heifers of breeding size (275-325 kg) and age (16-18 months) were used to study the uterine environment as affected by several management practices. Twelve dairy heifers in the Louisiana State University replacement herd (non-slaughter) were used in phase one of the study and 18 Holstein heifers available for slaughter were used in phase two. Uterine fluid was collected from live animals in both phase one and phase two on day 0 and day 2 of all the estrous cycles. In phase two, in addition to samples from live animals, samples of uterine fluid and physiological saline flushings of the oviduct of each animal were collected at the time of slaughter.

1. Phase One

Phase one was conducted during the months of July and August with the 12 heifers being divided, at random, into Group I and Group II. Uterine fluid was collected on day 0 and day 2 of the estrous cycle from heifers in Groups I and II as each exhibited estrus. Heifers in Group II were confined to a small paddock and individually fed MGA for estrus synchronization. The estrous synchronized heifers were fed individually 1.0 mg of MGA (0700 hours) in 0.91 kg of grain for 14 consecutive days in addition to daily grain and grass hay.

Heifers in Group I were on pasture and were fed supplemental silage and grain so that both groups were offered net energy and crude protein at 100% of the NRC recommended level for developing dairy heifers.

2. Phase Two

Phase two of this study began in October with 18 Holstein heifers that were available for slaughter at the termination of the experiment. Upon initiation of the study uterine fluid was collected from those heifers exhibiting non-synchronized estrus. The first 10 heifers to exhibit non-synchronized estrus were designated as Group III, and were the heifers to be placed in the environmental control chamber, under hot conditions (Table 1), for one estrous cycle. The remaining 8 heifers were assigned to Group IV and were maintained under cool conditions with maximum and minimum dry bulb temperatures of 21 C and 14 C, respectively. Both groups of heifers were fed MGA for estrus synchronization the same as described for phase one. Uterine fluid was collected on day 0 and day 2 of the estrous cycle, following non-synchronized estrus and again following the first estrus after withdrawal of MGA. After collection of uterine fluid on day 2 following the first estrus after withdrawal of MGA, the heifers in Group III were placed in an environmental control chamber simulating summer conditions in Louisiana for one estrous cycle. The conditions of the environmental control chamber for the 24 hour cycle are given in Table 1, with maximum and minimum dry bulb temperatures of 33.3 C and 24.4 C, respectively. These heifers (Group III) were slaughtered and uterine fluid and physiological saline flushings of the oviduct were collected

TABLE 1. The dry bulb temperature, dew point, relative humidity and vapor pressure used in the environmental control chamber to heat stress Group III.

Time	Dry bulb	Dew point	Relative humidity	Vapor pressure
	----------(°C)----------		--(%)---	(mm Hg)
0600	24.4	19.4	74	17.0
0800	27.8	21.7	69	19.3
1000	30.0	23.3	67	21.6
1200	32.2	25.0	65	23.6
1300	33.3	25.0	62	23.6
1500	33.3	24.4	60	23.1
1600	32.2	23.9	62	22.4
1800	30.6	22.8	64	20.1
2000	28.9	21.7	65	19.3
2200	25.5	19.4	69	17.0
2400	24.4	19.4	74	17.0
0600	Repeat of the 24 hour cycle			

following the estrus manifested while in the environmental control chamber. This was the second estrus after withdrawal of MGA and is the estrus when conception rate has been found to return to the expected percent, unless other conditions are imposed on the animals. Group IV, the remaining 8 heifers, were confined to a small paddock under natural fall and early winter conditions (maximum of 21 C and minimum of 14 C) of Louisiana through the second estrus after withdrawal of MGA, at which time these heifers were slaughtered and uterine fluid and a physiological saline flushing of the oviduct collected.

B. Collection of Uterine Fluid

1. From Live Animals

Uterine fluid was collected from live animals using a modification of a technique described by Fahning et al. (20). The collection

apparatus, as modified, is shown in Figures 1 and 2. Heifers were restrained in stanchions and a large plastic insemination tube, approximately 7 mm in outside diameter and 56 cm in length, was used as a cannula through the crevix. The inside diameter of the cannula was large enough to allow free passage of a polyethylene catheter, O.D. 2.41 mm by I.D. 1.68 mm, into the uterus. The original polyethylene catheter was approximately 112 cm in length with 6 holes cut into the wall of the end of the tubing to be placed in the uterus. The holes gave a larger surface area exposed to the uterus which aided in aspiration of the fluid. A silastic tubing tip with holes cut into the wall, with the same outside diameter and approximately 7.5 cm in length, was later placed on the end of the polyethylene tube by an inside metal connector. Silastic tubing was found to be more pliable and less harsh than polyethylene tubing and therefore caused less bleeding of the very sensitive endometrial wall of the uterus and uterine horn.

The anterior end of the insemination tube or cannula was beveled to facilitate insertion through the cervix of heifers. Cannulation of the cervix was accomplished by the rectal palpation technique used in artificial insemination. A catheter was inserted through the cannula so that the tip of the catheter would be in the body and/or horn of the uterus. The free end of the catheter was connected to a 40 ml vacuum-collection chamber by means of a #13 California bleeding needle placed through the rubber stopper used to seal the collection chamber. A 50 ml glass syringe, used to provide negative pressure for aspiration of the uterine fluid, was attached to the collection chamber by a short latex rubber hose connected to a hypodermic needle placed through the

Figure 1. The collection apparatus assembled.

Figure 2. The parts of the collection apparatus.

rubber stopper. A by-pass adapter was fitted on the glass syringe to allow the plunger of the syringe to be returned to the zero position without forcing fluid in the catheter back into the uterus. By this means more than one aspiration attempt could be made without forcing fluid, collected in prior aspiration attempts, from the catheter. Following collection, uterine fluid in the catheter was flushed from the catheter into the vacuum collection chamber by aspiration of 2 ml of physiological saline. The mixture was poured into a sample vial and the mixture again drawn by aspiration through the catheter in an effort to remove most of the viscous uterine fluid from the catheter. Uterine fluid samples were poured into sample vials, sealed, labeled, and stored at -10 C for later analysis.

Uterine fluid was collected from all animals on day 0 and day 2 of the estrous cycle. Day 0 was designated as 8 to 12 hours after the onset of estrus or standing heat; day 2 was 48 hours later. The time designated as day 0 was selected to coincide with the usual recommended time of insemination. Rectal temperature and respiration rates were taken prior to collection of uterine fluid on day 0 and day 2. A sample of blood was collected, by means of a jugular puncture, immediately after uterine fluid collection. Group III and Group IV were further divided into two parts the second estrus after withdrawal of MGA, and one-half of each group was slaughtered on day 0 and the remainder on day 2.

2. From Slaughtered Animals

The animals were slaughtered to coincide with day 0 and day 2, as designated for live animal collections. The animals were hauled to

a local abattoir for slaughter and the reproductive tracts were removed intact. Each tract was ligated at the vaginal-cervical junction to prevent any mixing of vaginal and uterine fluid before a sample of uterine fluid could be taken. After ligation the tracts were placed in individual plastic bags, sealed and placed in an ice chest. The reproductive tracts were returned to the Louisiana State University Dairy Science Physiology Laboratory in the ice chest and a sample of uterine fluid collected by aspiration. After the sample of uterine fluid was collected, in a manner similar to that used in the live animals, hemostats were placed at the utero-isthmic junction, isthmic-ampullary junction and at the fimbrial-ampullary junction. The oviduct was removed and sectioned into the ampulla and the isthmus and reverse flushed with 1.0 ml of physiological saline. A disposable 1 cc syringe, fitted with a 1 inch 25 guage needle, was used to flush the sections of the oviduct into separate containers. These flushings, as well as the sample of uterine fluid collected, were labeled and stored at -10 C for later analysis.

C. Environmental Control Chamber

The Louisiana State University environmental control chamber was used to simulate summer conditions in Louisiana (62,63) in an effort to heat stress heifers in Group III. Individual leather neck halters were used to restrain heifers in individual stalls of the chamber after collection of uterine fluid on day 2 following first estrus after withdrawal of MGA. The stalls were equipped with individual feed bunks and automatic watering cups that allowed ad libitum consumption

of water. Heifers were fed twice daily at 0730 and 1730 hours and continued on the same feeding program in the chamber as described earlier.

Heifers in the environmental control chamber were subjected to maximum temperatures of 29.5 C and a vapor pressure of 19.5 mm Hg and minimum temperatures of 24.4 C and a vapor pressure of 17.0 mm Hg for 2 days. During these 2 days there was a gradual increase in temperature and vapor pressure until the maximum was reached and these conditions were maintained for 5 hours. After the period of maximum conditions there was a gradual reduction in temperature and vapor pressure to minimum conditions which were maintained for the remaining part of the 24 hour cycle.

Two cycles with maximum temperature of 35 C and a vapor pressure of 23.8 mm Hg were attempted. These conditions caused extremely high respiration rates and therefore, each cycle was altered after the heifers had been exposed to about 2 hours of these conditions. Environmental control chambers maintained at 32.2 C or above with 50% or greater relative humidity have been used by several workers (38,39,62,63) to give what was considered to be "Hot" conditions or heat stress. A 24 hour cycle with maximum temperatures of 33.5 C and a vapor pressure of 23.6 mm Hg and minimum temperature of 24.4 C and a vapor pressure of 17.0 mm Hg was selected as being sufficient to heat stress aminals with winter hair coats. Specific conditions maintained in the chamber, for each 24 hour cycle, are given in Table I. Heifers in the environmental control chamber were under 14 hours of continuous light each day.

Beginning with the 15th day in the chamber, the heifers were turned outside as a group twice daily for estrus checks of approximately 30 minutes at 0700 hours and 1700 hours, until each animal was observed in standing estrus. After animals exhibited standing heat they were transported to the local abattoir for slaughter, on the schedule described earlier.

D. Laboratory Procedure

The fluid samples were removed from storage in groups of 12 and allowed to thaw. Protein concentration was determined using the biuret method as modified and described by Todd et al. (73). Samples were lyophilized and distilled water was used as a solvent in reconstituting the samples to a designated volume. Uterine fluid samples were reconstituted to 0.6 ml and the physiological saline flushing of the sections of the oviduct to 0.4 ml. Reconstituted samples of uterine and oviductal fluid were deproteinized with acetone, using a 1:1 ratio, and centrifuged at 250 X g for 10 minutes. The supernatant fluid was decanted into a test tube and the test tube placed into a constant temperature heat block, automatically controlled at 75 C, to remove the acetone by vaporization. The deproteinized samples were centrifuged at 300 X g for 10 minutes and decanted. Twenty five lambda of the reconstituted, deproteinized fluids were placed on pherogram paper and the pherogram placed in the high voltage electrophoresis cell of the CMAG unit. A modification of the procedure described by Atfield and Morris (2) was used for these samples. Electrophoretic separation was accomplished in a

formic-acetic-water buffer (26:120:1000) using 4500 DC volts for 16 minutes. These conditions were acceptable for separating most of the deproteinized samples into amino acid bands on the electropherogram. After electrophoresis, the electropherogram was suspended in a 100 C oven for 10 minutes to dry. The dry electropherogram was immersed in a 0.2% ninhydrin in acetone solution and returned to the oven for two minutes. The electropherogram was cut into strips and an analytrol (Beckman Spinco, Model RB) used to record on graph paper the density of the colored bands of amino acids present in each sample. The quantity of amino acids present in each sample was determined by comparing the density measurement of the sample with known concentration density measurements of pure amino acids.

E. Statistical Analysis

Analysis of variance, as described by Snedecor and Cochran (69), was conducted on the amounts of amino acids in the various treatment groups. There were seven animals common to day 0 and day 2 of non-synchronized estrus and the first estrus after withdrawal, and a randomized block 2X2 factorial analysis was conducted.

CHAPTER IV

RESULTS AND DISCUSSION

A. Protein Content of Uterine and Oviductal Fluid

A summary of the protein levels observed in uterine fluid is presented in Tables 2 and 3. Considerable variation in the protein level was observed between individuals within the same treatment group. The average protein content of uterine fluid was lower on day 0 than on day 2 in both non-synchronized (Group I) and synchronized (Group II) with an average level of 1.07 mg/ml for day 0 of Group I and 1.21 mg/ml for day 2. In Group II the average level was 0.97 mg/ml for day 0 and 1.68 mg/ml for day 2. Uterine fluid from Group III (Hot) had an average protein level of 0.88 mg/ml for day 0 and this was the lowest average protein content for any group collected from live animals. It should be pointed out that 4 of the 7 values were from animals slaughtered on day 0. Uterine fluid collected from reproductive tracts after stunning and exsanguination, in the slaughter process, contained lower levels of protein on both day 0 and day 2. Although all samples that contained noticeable amounts of blood were discarded, and are noted in Tables 2 and 3, samples from live animals contained, on the average, more protein than samples collected after slaughter. Explanation for this lower concentration of protein is not clear; however, it is possible that samples from live animals, considered to be typical uterine fluid, contained sufficient blood to cause an elevation in protein level.

TABLE 2. Protein content of uterine fluid from dairy heifers following non-synchronized and synchronized estrus the first estrus after withdrawal of MGA.

NON-SYNCHRONIZED			SYNCHRONIZED		
Animal number	Day 0	Day 2	Animal number	Day 0	Day 2
	-----mg/ml------			-----mg/ml------	
5	2.04	2.92	5	.60	1.48
6	.56	bl	6	.96	bl
8	.30	.76	8	.60	2.04
9	1.80	1.02	9	bl	bl
10	1.26	1.10	10	bl	.60
11	.60	1.10	I1	.30	bl
12	.90	1.48	12	1.48	1.10
14	.90	---	14	1.20	---
17	1.20	---	17	---	bl
18	.80	---	18	1.20	---
20	1.36	---	20	.68	bl
7	1.10	.80	1	1.14	bl
16	1.48	1.20	2	bl	2.40
918	1.20	bl	13	---	2.96
922	.56	bl	B21	1.80	1.20
935	1.00	.48	K71	1.20	bl
			879	.48	---
			926	1.20	---
			932	.56	---
			933	1.20	---
Avg.	1.07	1.21		.97	1.68
Range	.30-2.04	.48-2.92		.30-1.80	.60-2.04

bl Bloody sample
--- No sample available

TABLE 3. Protein content of uterine fluid and physiological saline flushings of the oviduct following the second estrus after withdrawal of MGA (Group III and IV).

Animal number	Uterine Fluid		Isthmus	Ampulla
	Day 0	Day 2		
	mg/ml			
GROUP III				
5	.20	---	.12[a]	.23[a]
6	.60	---	.06[a]	.09[a]
10	.42	---	.12[a]	.23[a]
11	1.92	.37	.06	.20
14	1.68	.34	.12	.06
16	.15	---	.06[a]	.40[a]
17	.37	---	.63[a]	.25[a]
18	bl	.78	.14	.25
20	1.68	.23	.14	.06
Avg.	.88	.43	.16	.20
Range	.15-1.92	.23-.78	.06-.63	.06-.40
GROUP IV				
1	.57	---	.14[a]	.25[a]
2	1.72	.51	.14	.15
4	.45	---	.15[a]	.20[a]
7	bl	.40	.23	.23
8	1.56	.60	.28	.14
12	.96	.60	.06	.28
Avg.	1.05	.53	.17	.21
Range	.45-1.72	.40-.60	.14-.28	.14-.28

[a] Values for day 0
bl Bloody sample
--- Slaughtered on day 0

Protein content of uterine fluid from rabbits has been reported to be 2.87 mg/ml (70) and uterine fluid from rats 3.49 mg/ml (58). The protein content of the oviductal fluid collected by cannulation in ewes has been reported to range from 9.4 to 28.6 mg/ml (50). According to Restall and Wales (57), the average protein content of oviductal fluid was 13.6 mg/ml for normal ewes and 39.6 mg/ml for spayed ewes. Secretions from cannulated oviduct of rabbits have been reported to contain 2.13 mg (34) and 2.73 mg (28) of protein per ml. The physiological saline flushings of the isthmus, collected on day 0 and day 2 of this investigation, contained an average of 0.16 and 0.17 mg/ml of protein for Groups III and IV, respectively (Table 3). Ampullary flushings of the same animals contained a slightly, although not significant, higher average protein content of 0.20 and 0.21 mg/ml for Groups III and IV, respectively. These values are lower than those reported by other investigators (28,34,50,57,58,70) in other species using continuous collection techniques; however, this was as expected due to the dilution effect. If the flushings had a dilution factor of 10 to 1, then these values would be in close agreement with those for rabbits (28,34) but considerably less than those values reported for fluid from the cannulated oviduct of sheep (53,57).

Uterine fluid samples containing blood, in sufficient concentration to be detected by the eye, were discarded. In Table 2 it can be seen that Group II (synchronized) had more bloody samples than any of the other groups (Table 3). Whether this increase in blood in the uterus is associated with the first estrus after withdrawal of MGA and other

progestogens (68) used for estrus synchronization, needs further investigation. It is possible that the presence of blood in the reproductive tract, the first estrus after withdrawal of MGA, is contributing to the unfavorable uterine environment that is responsible for the lower reproductive efficiency associated with estrus synchronization.

B. Amino Acids in Uterine Fluid

A summary of the number and quantity of amino acids detected in uterine fluid of dairy heifers in Group I, on day 0 following non-synchronized estrus, is presented in Tables 4 and 5. Seven amino acids were detected in 75% or more of the uterine fluid samples collected on day 0 which included unknown 1, glutamic acid, threonine, leucine-isoleucine, alanine, glycine and arginine. Three amino acids, valine, lysine and histidine, occurred less frequently than the seven listed above, combining, however, to give a total of 10 amino acids detected in 50% or more of the uterine fluid samples for day 0. Other amino acids detected in less than 50% of the uterine fluid samples are presented in Table 5 and were aspartic acid, phenylalanine, serine and unknown 2.

The average and range of values for each amino acid is given in Tables 4 and 5 for Group I. There was considerable variation in amount of amino acids in uterine fluid of individual animals. The number of samples available for each day was limited and no differences were detected statistically between day 0 and day 2 for the level of amino acids that occurred in 75% or more of the uterine fluid samples. The probability of detecting small differences is low in samples of the size of

TABLE 4. Amino acids detected most frequently in uterine fluid of dairy heifers on day 0 following non-synchronized estrus

Animal number	Unknown #1	Glutamic acid	Threo-nine	Leucine[a]	Alanine	Glycine	Arginine	Lysine
	mcg/ml							
882	8.4	17.6	---	3.2	7.2	5.1	27.8	15.4
922	20.8	trace	8.2	trace	trace	trace	---	---
927	41.8	---	46.5	76.4	36.0	43.3	44.5	---
5	75.2	60.5	68.4	17.0	18.7	22.9	33.4	9.2
6	75.2	48.2	38.3	---	8.6	28.0	22.3	18.4
7	37.6	trace	24.6	33.9	10.1	15.3	61.3	---
8	53.2	65.5	63.6	---	11.5	13.4	23.7	9.2
9	25.1	30.2	38.3	6.4	8.6	17.8	39.0	---
10	66.8	52.9	73.9	8.5	23.0	39.4	---	---
11	75.2	---	24.6	55.2	17.3	25.4	27.8	18.3
12	20.9	115.2	138.2	21.2	25.2	50.9	172.6	20.0
14	58.5	43.4	30.1	78.5	25.2	57.2	91.9	---
16	62.6	80.6	60.2	17.0	15.8	25.4	50.1	52.2
17	100.3	80.6	49.3	127.2	27.4	68.7	33.4	33.8
18	---	30.2	24.6	---	7.2	7.6	50.1	---
20	91.9	100.8	142.7	---	31.7	43.7	72.4	---
Avg.[b]	54.2	52.1	55.4	42.2	17.2	29.1	53.6	22.1
Range	8.4-100.3	17.6-115.2	8.2-142.7	3.2-127.2	2.0-36.0	2.0-68.7	22.3-172.6	9.2-52.2

[a]Leucine and isoleucine are reported as one value

[b]Average based on number of samples containing the amino acid

--- Amino acid was not detected

Trace Averaged as 2.0 mcg/ml

TABLE 5. Amino acids detected less frequently in uterine fluid of dairy heifers on day 0 following non-synchronized estrus.

Animal number	Aspartic acid	Phenyl-alanine	Serine	Valine	Unknown #2	Histidine
	----------	----------	----------	mcg/ml ----------	----------	----------
882	---	---	---	9.5	---	---
927	---	---	---	---	91.9	38.3
5	---	---	25.3	---	---	16.4
6	---	---	---	25.3	---	---
7	---	---	---	---	29.3	10.9
8	21.4	trace	---	31.7	15.7	15.1
9	---	---	---	22.2	---	21.9
10	---	---	37.9	---	60.1	---
11	---	---	---	---	75.2	16.4
12	---	---	88.7	15.8	---	54.7
14	---	50.2	---	---	112.8	38.3
16	---	---	46.5	31.7	41.8	---
17	---	---	---	107.7	---	---
18	---	---	---	9.5	---	---
20	---	---	19.0	79.2	33.4	---
Avg.[a]	21.4	26.1	43.5	37.0	57.5	26.5
Range		2.0-50.2	19.0-88.7	9.5-107.7	15.7-112.8	10.9-54.7

[a]Average based on number of samples containing the amino acid

--- Amino acid was not detected

Trace Averaged as 2.0 mcg/ml

Group I and where the variation among individuals is as large as found in this group.

Alanine was the lowest in average concentration of any of the amino acids in Group I for day 0, with 17.2 mg/ml and unknown 2 had the highest average level of 57.5 mg/ml (Tables 4 and 5). The range and quantity of amino acids detected in Group I was from a trace (considered to be 2.0 mg/ml), for several amino acids, to a high of 172.6 mg/ml for arginine in uterine fluid from animal number 12.

A typical migration pattern of pure amino acids on the electropherogram, as recorded on graph paper by the analytrol, is shown in Figure 3a. The typical pattern of amino acids in uterine fluid of Group IV (as recorded from an electropherogram strip) is shown in Figure 3b and a representation pattern for Groups I, II, and III in Figure 3c. Unknown 1 was an amino acid or nitrogen compound that did not migrate from the base line (Figure 3c) or place of sample application on the pherogram. Unknown 2 migrated on the electropherogram to a place that was between valine and alanine (Figure 3c). Neither of these was identified when compared with 20 pure amino acids and 3 other nitrogen compounds (Appendix Table 8). Unknowns 1 and 2 and the other amino acids were not from blood in the uterine fluid samples; whole blood and serum were analyzed by the same technique used for uterine fluid and no amino acid bands were detected on the electropherogram. The deproteinized components of blood did not migrate under the buffer conditions used for uterine fluid.

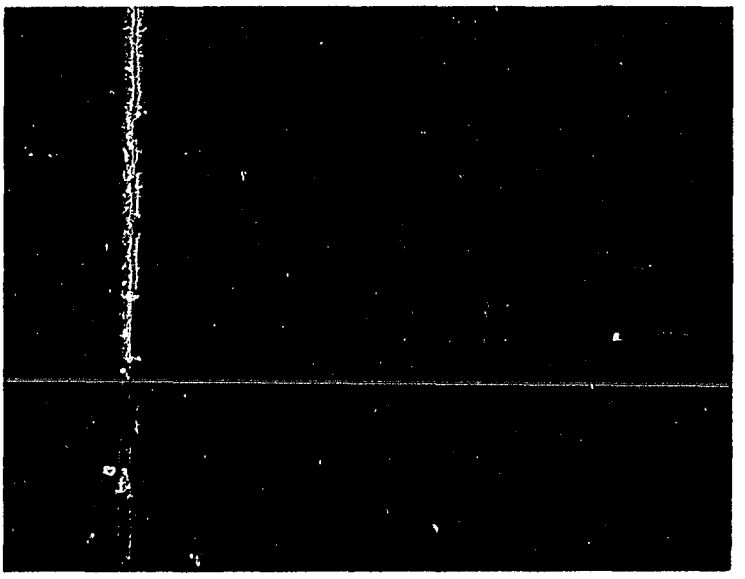

Figure 3a. The migration pattern of pure amino acids.

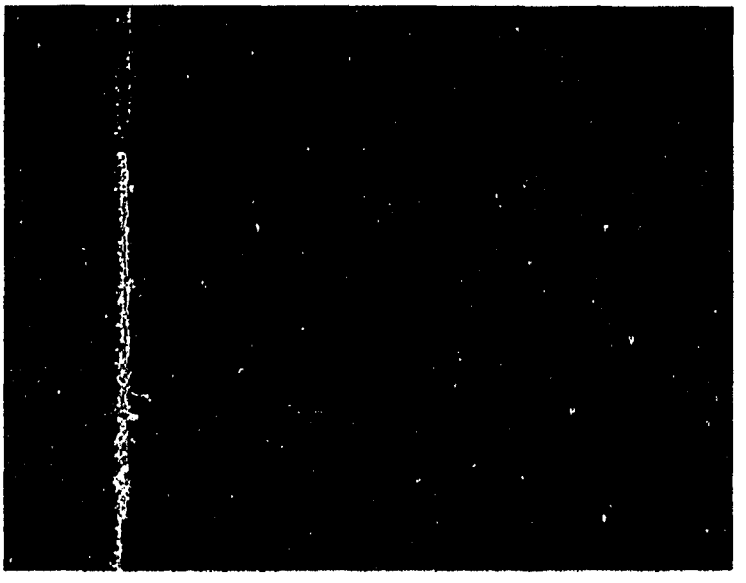

Figure 3b. A typical migration pattern of amino acids in uterine fluid of Group IV.

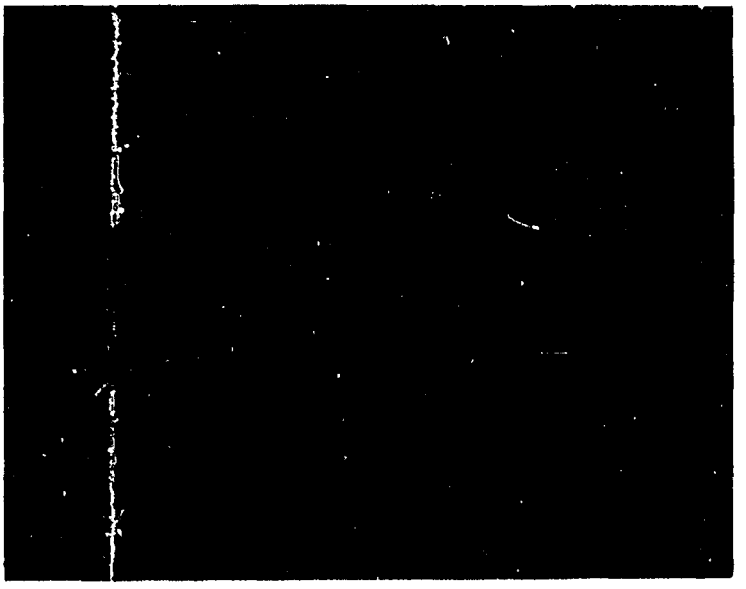

Figure 3c. A Sample that is representative of Groups I, II, and III showing the locations of unknowns 1 and 2.

Amino acids detected on day 2 following non-synchronized estrus were similar to those for day 0 and are presented in Appendix Tables 1 and 2. Amino acid levels (Table 6) in uterine fluid varied between day 0 and day 2 but no significant differences or trends were observed. Aspartic acid appeared in only one sample on day 0 and the presence of phenylalanine in only 3 samples indicates that these amino acids are not the primary amino acids needed for embryonic development. The effects of excessive amino acids and/or concentration in uterine fluid on blastocyst development are not known. The total amount of amino acids in uterine fluid showed considerable variation among animals within groups. The total amount of amino acids in uterine fluid is presented in Appendix Table 3.

Fourteen amino acids were detected in uterine fluid from Group I and a summary of the number, amount and ratio of amino acids is presented in Table 6. Four amino acids, unknown 1, glutamic acid, alanine and glycine occurred in 75% or more of the samples for both day 0 and day 2, whereas, threonine, leucine-isoleucine and arginine were also detected in 75% or more of the samples for day 0. The rather drastic drop in the number of samples containing histidine on day 2, one out of 13 samples, as compared to 8 out of 16 samples for day 0, is unexplained. A similar, although less drastic drop was noted for unknown 2 and lysine, which decreased from 8 out of 16 samples for day 0 to 3 out of 13 samples for day 2.

Fifteen amino acids were detected in uterine fluid collected on day 0 and day 2 following the first estrus after withdrawal of MGA

TABLE 6. The number, amount and ratio of amino acids in uterine fluid of non-sychronized dairy heifers.

Amino acid	Day 0			Day 2		
	Average[e]	Range	Ratio[c]	Average	Range	Ratio
	----(mcg/ml)----			---(mcg/ml)---		
Unknown (1)	54.2[a]	8-100	15/16	47.6[b]	2-125	12/13
Aspartic acid	21.4	---	1/16	0.0	---	---
Phenylalanine	26.1	2-50	2/16	70.9	---	1/13
Glutamic acid	52.1[a]	17-115	14/16	46.7[b]	2-106	13/13
Threonine	55.4[a]	8-143	15/16	64.0	25-129	8/13
Leucine[d]	42.2[a]	3-127	12/16	18.4	2-47	6/13
Serine	43.5	19-89	5/16	35.2	15-66	3/13
Valine	37.0	10-108	9/16	41.2	10-98	5/13
Unknown (2)	57.5	16-113	8/16	12.5	2-31	3/13
Alanine	17.2[a]	2-36	16/16	15.8[b]	6-37	12/13
Glycine	29.1[a]	2-69	16/16	25.1[b]	3-87	10/13
Histidine	26.5	11-55	8/16	38.3	---	1/13
Arginine	53.6[a]	22-173	14/16	62.4	6-240	8/13
Lysine	22.1	9-52	8/16	95.1	31-184	3/13

[a]Amino acids occurring in 75% or more of the uterine fluid samples on day 0.

[b]Amino acids occurring in 75% or more of the uterine fluid samples on day 2.

[c]Ratio of samples containing the amino acid to the number of samples collected.

[d]Leucine and isoleucine are reported as one value.

[e]Average based on number of samples containing the amino acid.

MGA (Group II), and these results are summarized in Table 7. Nine amino acids occurred in 50% or more of the uterine fluid samples collected on day 0: unknown 1, glutamic acid, threonine, leucine-isoleucine, valine, alanine, glycine, arginine and lysine. Ten amino acids were detected in uterine fluid samples for day 2, with serine being added to the 9 amino acids listed for day 0. The major difference in Group II, in comparison to Group I, was the detection of taurine (2/16 samples, day 0 and 3/15 samples, day 2) and ornithine (3/16 samples, day 0 and 4/15 samples, day 2; Table 7). Another difference was that aspartic acid did not appear in any of the uterine fluid samples of Group II. Individual results for Group II, for both days of collection, are presented in Appendix Tables 4, 5, 6 and 7. The significance of the presence of taurine and ornithine is not known but could be associated with the feeding of MGA for estrus synchronization and/or the effect of heat stress. Heifers in Group I were under natural heat stress and uterine fluid from these heifers did not contain these two amino acids; however, one sample contained aspartic acid.

The data for Group II were analyzed statistically the same as Group I. No differences were detected in level of amino acid for day 0 and day 2; however, the probability of detecting a small difference is extremely small in samples of the size of Group II and where variation among individuals is as large as exist in this group. A Student "t" test was used to compare the means of day 0 of Groups I and II, as well as means for day 2 for these groups. No significant differences were detected due to the large standard error for these groups.

TABLE 7. The number, amount and ratio of amino acids in uterine fluid of dairy heifers following the first estrus after withdrawal of MGA.

Amino acid	Day 0			Day 2		
	Average[e]	Range	Ratio[c]	Average	Range	Ratio
	----(mcg/ml)----			----(mcg/ml)---		
Unknown (1)	90.9[a]	2-347	14/16	128.0[b]	4-347	15/15
Taurine	66.2	58-75	2/16	111.3	23-207	3/15
Phenylalanine	17.7	---	1/16	88.6	---	1/15
Glutamic acid	31.3[a]	13-91	13/16	77.4[b]	13-141	11/15
Threonine	51.4[a]	2-120	15/16	82.1[b]	3-192	13/15
Leucine[d]	97.5	4-213	8/16	85.0	2-264	8/15
Serine	7.6	2-16	4/16	44.6	2-148	8/15
Valine	30.1	3-82	9/16	80.3	3-253	9/15
Unknown (2)	126.1	7-221	5/16	99.2	13-255	4/15
Alanine	19.4[a]	2-52	16/16	30.2[b]	2-141	15/15
Glycine	36.5[a]	2-127	16/16	60.3[b]	5-191	15/15
Histidine	40.8	7-77	5/16	52.8	49-55	3/15
Arginine	58.1[a]	6-212	13/16	87.4[b]	2-239	14/15
Lysine	29.5	9-68	9/16	40.1[b]	12-111	11/15
Ornithine	43.6	16-58	3/16	62.4	19-86	4/15

[a]Amino acids occurring in 75% or more of the uterine fluid samples on day 0.

[b]Amino acids occurring in 75% or more of the uterine fluid samples on day 2.

[c]Ratio of samples containing the amino acid to the number of samples collected.

[d]Leucine and isoleucine are reported as one value.

[e]Average based on number of samples containing the amino acid.

Groups III and IV contained fewer samples than Groups I and II and were not subjected to the above analysis.

Unknown 1, glutamic acid, threonine, alanine, glycine and arginine occurred in 75% or more of the uterine fluid samples collected from Group II on both day 0 and day 2. Lysine was also detected in 75% of the samples on day 2. This is of interest in that the appearance of lysine in uterine fluid of Group I decreased drastically on day 2, but in Group II the occurrence of lysine increased on day 2. The significance of this is not known but could be related to the use of MGA for estrus synchronization and, therefore, with lower conception rate in animals the first estrus after withdrawal.

Seven animals were in both Group I and Group II and a randomized block 2X2 factorial analysis was conducted (see Appendix Table 7). No differences were detected between days or between treatments (non-synchronized versus synchronized) and no significant interaction observed.

Twelve amino acids detected in uterine fluid from dairy heifers in Group III (2nd estrus after withdrawal, Hot) are summarized in Table 8 and individual results are given in Appendix Tables 8 and 9. Amino acids detected in 75% or more of the uterine fluid samples for both day 0 and day 2 were unknown 1, threonine, serine, alanine, glycine and arginine, while valine and taurine were also present in 75% or more of the uterine fluid samples for day 2. It can be seen, from summary Table 8, that taurine was present in 2 out of 10 samples on day 0 and in 3 out of 4 samples for day 2 and that ornithine was not

TABLE 8. The number, amount and ratio of amino acids in uterine fluid of dairy heifers under hot condition following the second estrus after withdrawal of MGA.

Amino acid	Day 0			Day 2		
	Average[e]	Range	Ratio[c]	Average	Range	Ratio
	----(mcg/ml)----			----(mcg/ml)---		
Unknown (1)	114.8[a]	42-238	9/10	139.8[b]	54-238	4/4
Taurine	126.7	121-132	2/10	249.6[b]	92-334	3/4
Glutamic acid	89.2	48-151	5/10	95.7	65-126	2/4
Threonine	74.8[a]	22-140	10/10	109.4[b]	93-126	3/4
Leucine[d]	108.7	10-213	5/10	40.1	21-59	2/4
Serine	32.0[a]	17-66	9/10	36.6[b]	19-53	3/4
Valine	67.6	38-86	3/10	82.4[b]	63-101	3/4
Unknown (2)	175.4	---	1/10	0.0	---	---
Alanine	34.0[a]	12-53	10/10	40.0[b]	32-52	4/4
Glycine	51.7[a]	13-97	10/10	57.9[b]	38-92	4/4
Arginine	72.4[a]	22-111	8/10	103.0[b]	56-161	4/4
Lysine	15.3	9-22	2/10	0.0	---	---

[a]Amino acids occurring in 75% or more of the uterine fluid samples on day 0.

[b]Amino acids occurring in 75% or more of the uterine fluid samples on day 2.

[c]Ratio of samples containing the amino acid to the number of samples collected.

[d]Leucine and isoleucine are reported as one value.

[e]Average based on number of samples containing the amino acid.

detected in any of the samples for Group III. It was further observed that unknown 2 and lysine were not detected in any samples collected on day 2.

A summary of the nine amino acids detected in uterine fluid collected on both day 0 and day 2 from Group IV (2nd estrus after withdrawal, Cool) is given in Tables 9 and 10. Six amino acids, glutamic acid, threonine, serine, alanine, glycine and arginine, were detected in 75% or more of the uterine fluid samples for both day 0 and day 2. Lysine was observed in 5 out of 6 samples of uterine fluid collected on day 0, but none of the uterine fluid samples on day 2 contained lysine and valine was detected only in uterine fluid from animal 12 on day 0. Leucine-isoleucine was detected in only 2 out of 6 samples for day 0 and in 1 out of 4 samples for day 2. Five amino acids, glutamic acid, threonine, alanine, glycine and arginine, were detected in 100% of the uterine fluid samples. Serine did not appear in the samples of uterine fluid collected from animal number 7 on either day of collection.

Amino acids in the physiological saline flushings of the ampulla and the isthmus of Group IV are given in Table 11. Four of the amino acids, appearing in 100% of the uterine fluid samples of Group IV, were consistently present in both the ampullary and the isthmic flushings: glutamic acid, threonine, alanine, and glycine. The fifth amino acid, arginine, was detected in 3 out of 12 flushing samples while leucine-isoleucine and serine were present in over 50% of the flushings from both the ampulla and the isthmus. Comparing these findings with the

TABLE 9. Amino acids in the uterine fluid of dairy heifers on day 0 and day 2 following the second estrus after withdrawal of MGA (Group IV).

Animal number	Glutamic acid	Threonine	Leucine[c]	Serine	Alanine	Glycine	Arginine	Lysine
	mcg/ml							
DAY 0								
1	30.2	24.6	---	8.5	11.5	15.3	11.1	12.3
2	7.6	23.3	---	8.5	5.8	11.5	19.5	---
4[b]	17.6	91.7	---	37.0	36.0	19.1	8.4	18.4
7	76.6	207.0	---	---	28.8	61.1	trace	trace
8	21.4	30.1	43.3	15.8	20.9	57.9	39.0	8.5
12[a]	26.5	39.7	7.4	16.4	9.0	18.4	16.7	16.9
Avg.[d]	30.0	69.4	25.3	17.2	18.7	30.5	12.8	11.6
Range	7.6-76.6	23.3-207.0	7.4-43.3	8.5-37.0	5.8-36.0	11.5-61.1	2.0-39.0	2.0-18.4
DAY 2								
2	15.1	30.1	---	12.7	4.3	10.2	22.3	---
7	206.4	221.8	---	---	80.6	114.5	261.8	---
8	30.2	38.3	---	8.4	14.4	12.7	trace	---
12	60.5	93.0	10.7	35.9	17.3	15.3	33.4	---
Avg.	77.9	95.8	10.7	19.0	29.2	38.2	79.9	---
Range	15.1-206.4	30.1-221.8	---	8.4-35.9	4.3-80.6	10.2-114.5	22.3-261.8	---

[a]Sample contained 24.6 mcg/ml of valine.

[b]Animal was slaughtered the last day of the experiment without exhibiting estrus and by ovary examination classified as day -1.

[c]Leucine and isoleucine are reported as one value.

[d]Zero values not included in the average

--- Amino acid was not detected.

TABLE 10. The number, amount and ratio of amino acids in uterine fluid of dairy heifers under cool condition following the second estrus after withdrawal of MGA.

Amino acid	Day 0			Day 2		
	Average[e]	Range	Ratio[c]	Average	Range	Ratio
	----(mcg/ml)----			-----(mcg/ml)---		
Glutamic acid	30.0[a]	8-77	6/6	78.0[b]	15-206	4/4
Threonine	69.4[a]	23-207	6/6	95.8[b]	30-222	4/4
Leucine[d]	25.3	7-43	2/6	10.7	---	1/4
Serine	17.2[a]	9-37	5/6	19.0[b]	8-36	3/4
Valine	24.6	---	1/6	0.0	---	---
Alanine	18.7[a]	6-36	6/6	29.2[b]	4-81	4/4
Glycine	30.5[a]	12-61	6/6	38.2[b]	10-115	4/4
Arginine	12.8[a]	2-39	6/6	79.9[b]	22-262	4/4
Lysine	11.6[a]	2-18	5/6	0.0	---	---

[a]Amino acids occurring in 75% or more of the uterine fluid samples on day 0.

[b]Amino acids occurring in 75% or more of the uterine fluid samples on day 2.

[c]Ratio of samples containing the amino acid to the number of samples collected.

[d]Leucine and isoleucine are reported as one value.

[e]Average based on number of samples containing the amino acid.

TABLE 11. Amino acids in the physiological saline flushing of the ampulla and isthmus of dairy heifers on day 0 and day 2 following the second estrus after withdrawal of MGA (Group IV)

Animal number	Glutamic acid	Threonine	Leucine[b]	Serine	Valine	Alanine	Glycine	Arginine
	mcg/ml[a]							
Ampullary Flushing Day 0								
1	---	11.4	12.3	5.3	---	12.0	21.2	---
4	25.2	63.8	5.3	8.8	---	14.4	21.2	---
Avg.[c]	25.2	37.6	8.3	7.1	---	13.2	21.2	---
Ampullary Flushing Day 2								
2	16.8	23.4	---	7.0	---	9.6	14.8	---
7	---	27.4	29.9	---	31.7	25.2	57.2	---
8	21.0	27.4	---	8.8	---	10.8	40.3	---
12	8.4	150.5	19.4	51.0	---	73.2	133.6	55.7
Avg.	15.4	57.2	24.6	22.3	31.7	29.7	61.5	55.7
Isthmic Flushing Day 0								
1	---	trace	---	trace	---	trace	trace	---
4	33.6	38.8	---	10.6	---	7.2	19.1	23.2
Avg.	33.6	20.4	---	6.3	---	4.6	10.6	23.2
Isthmic Flushing Day 2								
2	16.8	18.2	---	7.0	---	3.6	8.5	---
7	---	36.5	35.2	---	29.0	36.0	42.4	---
8	54.6	54.7	---	24.6	---	13.2	31.8	32.5
12	---	11.4	19.4	12.3	---	7.2	14.8	---
Avg.	35.7	30.2	27.3	14.6	29.0	15.0	24.4	32.5

[a]One ml of flushing concentrated to 0.4 ml.
[b]Leucine and isoleucine are reported as one value
[c]Average based on number of samples containing the amino acid
--- Amino acid was not detected

physiological saline flushings of the ampulla and the isthmus of Group III (Hot), in Tables 12 and 13, it will be noted that unknown 1 was detected in more than 50% of the ampullary flushings for day 0 and day 2. Also unknown 1 was observed in the flushings obtained from the isthmus on two occasions. Arginine was detected in 7 out of the 10 ampullary flushings on day 0 and day 2 but in only 2 out of the 10 isthmic flushings, whereas, in Group IV arginine was found in 1 out of the 6 ampullary flushings and 2 out of the 6 isthmic samples. The other amino acids, glutamic acid, threonine, leucine-isoleucine, serine, alanine and glycine, were present in a similar number of occasions in both Groups III and IV.

A summary of the amino acids occurring in the 4 treatments, as well as the totals for the study, are presented in Table 14. The frequency of occurrence of amino acids in uterine fluid of dairy heifers is summarized, for each group and for each collection day, in Table 15.

Animals fed MGA (60,61) or other progestogens (30) for estrus synchronization are reported to have normal or improved conception rate the second estrus after withdrawal. Group IV represents such a group of animals and, therefore, should have uterine and oviductal fluid compatable with satisfactory conception. In Group IV, considered to represent a management system that would result in a good conception rate, glutamic acid, threonine, serine, alanine, glycine and arginine were consistently found in uterine fluid. These amino acids plus leucine-isoleucine and valine were observed in the ampullary flushings

TABLE 12. Amino acids in the physiological saline flushing of the ampulla and isthmus of dairy heifers on day 0 following the second estrus after withdrawal of MGA (Group III).

Animal number	Unknown #1	Glutamic acid	Threonine	Leucine[c]	Serine	Alanine	Glycine	Arginine
	mcg/ml[a]							
Ampullary Flushing								
5[b]	55.7	29.4	75.2	3.5	4.9	31.2	53.0	6.4
6	13.9	12.6	16.0	---	1.8	8.4	25.4	---
9	---	---	18.2	---	21.1	13.2	17.0	37.1
10	38.3	21.0	68.4	3.5	5.3	25.2	59.4	9.3
16	---	79.8	79.8	17.6	26.4	36.0	61.5	37.1
17	114.8	58.8	36.5	109.1	38.7	60.0	86.9	27.8
Avg.[d]	55.7	40.3	47.3	33.4	16.4	20.0	50.5	23.5
Isthmic Flushing								
5	---	---	13.7	---	---	7.2	12.7	---
6	---	---	4.6	---	---	3.6	8.5	---
9	---	105.0	79.8	17.6	35.2	30.0	63.6	---
10	107.8	134.4	63.8	---	---	30.0	42.4	---
16	---	---	18.2	---	12.3	6.0	10.6	23.2
17	---	50.4	86.6	---	22.9	21.6	31.8	32.5
Avg.	107.8	96.6	44.4	17.6	23.5	16.1	28.3	27.8

[a]One ml of flushing concentrated to 0.4 ml.

[b]Sample contained 2.56 mcg of Lysine.

[c]Leucine and isoleucine are reported as one value.

[d]Average based on number of samples containing the amino acid.

--- Amino acid was not detected.

TABLE 13. Amino acids in the physiological saline flushing of the ampulla and isthmus of dairy heifers on day 2 following the second estrus after withdrawal of MGA (Group III)

Animal number	Unknown #1	Glutamic acid	Threo-nine	Leucine[b]	Serine	Alanine	Glycine	Arginine
	mcg/ml[a]							
Ampullary Flushing								
11	10.4	16.8	31.9	---	trace	8.4	17.0	---
14	---	---	13.7	---	---	3.6	8.5	trace
18	27.8	---	41.0	14.7	21.1	30.0	61.5	18.7
20	---	33.6	36.5	---	10.6	14.4	29.7	---
Avg.[c]	19.1	25.2	30.8	14.7	11.2	14.1	29.2	10.3
Isthmic Flushing								
11	17.4	---	16.0	---	4.9	4.8	8.5	---
14	---	8.4	20.5	---	1.8	12.0	31.8	---
18	---	---	---	15.8	---	4.8	12.7	---
20	---	12.6	20.5	---	5.3	10.8	17.0	---
Avg.	17.4	10.5	19.0	15.8	4.0	8.1	17.5	---

[a]One ml of flushing concentrated to 0.4 ml.

[b]Leucine and isoleucine are reported as one value

[c]Zero values not included in the average

--- Amino acid not detected

TABLE 14. A summary of the amino acids occurring in the four treatments.

Animo acid	Groups I	II	III	IV	Totals
	----------Ratio[b]----------				
Unknown (1)	27/29	29/31	13/14	0	69/84
Taurine	-	5/31	5/14	0	10/84
Aspartic acid	1/29	0	0	0	1/84
Phenylalanine	3/29	2/31	0	0	5/84
Glutamic acid	27/29	24/31	7/14	10/10	68/84
Threonine	23/29	28/31	13/14	10/10	74/84
Leucine[a]	18/29	16/31	7/14	3/10	44/84
Serine	8/29	12/31	12/14	8/10	40/84
Valine	14/29	18/31	6/14	1/10	29/84
Unknown (2)	11/29	9/31	1/14	0	21/84
Alanine	28/29	31/31	14/14	10/10	83/84
Glycine	26/29	31/31	14/14	10/10	81/84
Histidine	9/29	8/31	0	0	17/84
Arginine	22/29	27/31	12/14	10/10	71/84
Lysine	11/29	20/31	2/14	5/10	38/84
Ornithine	0	7/31	0	0	7/84

[a]Leucine and isoleucine are reported as one value.

[b]Ratio of the samples containing the amino acid to the number of samples collected.

TABLE 15. Amino acids detected most frequently in uterine fluid samples for each treatment.

	Groups							
	I		II		III		IV	
	Days							
Amino acid	0	2	0	2	0	2	0	2
Unknown (1)	+	+	+	+	+	+		
Taurine						+		
Aspartic acid								
Phenylalanine								
Glutamic acid	+	+	+	+	0	0	+	+
Threonine	+	0	+	+	+	+	+	+
Leucine[a]	+	0	0	0	0	0		
Serine				0	+	+	+	+
Valine	0		0	0		+		
Unknown (2)	0							
Alanine	+	+	+	+	+	+	+	+
Glycine	+	+	+	+	+	+	+	+
Histidine	0							
Arginine	+	0	+	+	+	+	+	+
Lysine	0		0	+			+	
Ornithine								

[a]Leucine and isoleucine are reported as one value.
0 Observed in 50% or more of the uterine fluid samples.
+ Observed in 75% or more of the uterine fluid samples
Group I - Non-synchronized estrus.
Group II - Synchronized estrus, first estrus after withdrawal.
Group III - Synchronized estrus, second estrus after withdraw Hot Group.
Group IV - Synchronized estrus, second estrus after withdrawal Cool Group.

of Group IV and valine and arginine were observed only in day 2 flushings. Further evidence is needed to determine whether these amino acids are required by the blastocysts, It is of interest to note that Gwatkins (25) indicated that in rats, arginine, histidine, leucine and threonine are among those amino acids "shown to be absolute requirements for outgrowth of the blastocyst in vitro." The timing of the appearance or disappearance of amino acids in the uterine fluid may be important. Butcher et al. (11) in 1969 concluded that a delay in ovulation produces changes in the intrauterine environment and that these changes lead to a decreased implantation rate. The importance of amino acids in blastocyst development has been demonstrated by Gulyas and Daniel (24) in mink, armadillo, fur seal, and rodent. These workers demonstrated the incorporation of labeled amino acids into these blastocysts and, also, quoted personal communication with Weithlauf as having shown secretion of amino acids with uterine fluid. Incorporation of these secreted amino acids was terminated in normal blastocysts when they were transferred to uteri of pseudo-pregnant, progesterone-treated, "delaying" animals. These results also indicate a hormonal effect on the developing blastocysts, directly or indirectly, through the environment of the blastocyst. Short and Yoshinga (67) reported that proper uterine environment was needed for implantation in rats and that even tumor cells would implant under hormonal conditions that permitted blastocyst implantation.

Uterine fluid from Group II, of the present study, contained six amino acids that were not detected in uterine fluid from animals in

Group IV (Table 14). Undernutrition has received much attention but excess or over-nutrition has not been so extensively studied. Toxicity of excessive amino acids is known (41), and it is possible that the presence of six additional amino acids in uterine fluid of heifers the first estrus after withdrawal of MGA could be exhibiting a toxic effect and reducing embryo survival. The presence of unknown 1, taurine and unknown 2 in uterine fluid from Group III but not in Group IV could be due to heat stress and have a detrimental effect on embryo survival. Perhaps the lower reproductive efficiency associated with both heat stress and synchronization are a result of an upset of the delicate ratio of hormones of the ovaries and adrenal cortex that are required for successful gestation (75).

During collection of uterine fluid a subjective evaluation was made of the characteristic "uterine tone" of the cervix and uterus; in approximately 40-50% of the heifers in Group II on day 0 the characteristic uterine tone was not detected. A more typical cervix and uterus for day 0 was detected on day 2 in more than half of the animals that lacked uterine tone on day 0. Cervix tone is mentioned by Roark and Herman (59) as a reliable indication of estrus. The observed delay in appearance of uterine tone in Group II may be a result of the failure of exogenous hormones to completely control the reproductive system (37) and this could account for the psychic manifestation of estrus before the uterus is in proper condition or tone.

These observations have been made on a small number of animals and further work is needed to confirm these findings. According to

results reported by McLachlan et al. (49) working with rabbits, evidence of this nature after mating is needed. It was further suggested that, during the pre-implantation stages of pregnancy, the uterine glands produce a secretion which is different in some respects from that secreted by the endometrium of nonpregnant does. In apparent opposition to this, mating was found to have no effect on protein content (50) or protein and peptide content (46) in sheep and rabbits, respectively.

CHAPTER V

SUMMARY AND CONCLUSIONS

Thirty nulliparous dairy heifers of breeding size (275-325 kg) and age (16-18 months) were used to study the effects of 4 different management systems on protein and amino acid content of uterine and oviductal fluids. Groups were identified according to the type of management the dairy heifers received: Group I, non-synchronized estrus and under natural summer conditions of Louisiana; Group II, synchronized estrus and under natural summer conditions of Louisiana; Group III, second estrus after withdrawal of MGA and maintained in an environmental control chamber for one estrous cycle under hot contitions (simulating condition of Groups I and II); Group IV, second estrus after withdrawal of MGA and maintained under cool conditions of late fall and early winter of Louisiana.

Uterine fluid was collected from all dairy heifers that exhibited estrus during this study. Uterine fluid and physiological saline flushings of the oviduct were collected from 18 heifers which were slaughtered at the termination of the experiment. Uterine fluid and oviductal flushings were collected on day 0 (8-12 hours after onset of standing estrus) and day 2 (48 hours after day 0). Protein and amino acid levels were determined for uterine fluid and physiological saline flushings from each group.

Average protein content of uterine fluid was lower on day 0 than on day 2 for Groups I and II; however, no significant differences were detected. Uterine fluid from reproductive tracts removed at slaughter consistently contained less protein than the samples collected from live animals but these differences were not statistically significant. The lower protein level in uterine fluid from Groups III and IV appeared to be due to the inclusion of protein values from animals slaughtered in each of these groups. The physiological saline flushings of the isthmus contained an average of 0.16 and 0.17 mg of protein per ml for Groups III and IV, respectively. The average protein level for ampullary flushings was 0.20 and 0.21 mg/ml for Groups III and IV, respectively.

Uterine fluid from heifers in Group IV, maintained under a more desirable management system, contained the following amino acids: glutamic acid (in 10 out of 10 samples, i.e., 10/10), threonine (10/10), alanine (10/10), glycine (10/10), arginine (10/10), serine (8/10), lysine (5/10), leucine-isoleucine (3/10), and valine (1/10). Three additional amino acids were detected in uterine fluid from Group III: unknown 1 (13/14), taurine (5/14) and unknown 2 (1/14). Amino acids detected, in Groups III and IV, were not observed in the same frequency; however, there was general agreement with glutamic acid, threonine, serine, alanine, glycine and arginine being present in 50% or more of the samples in these groups.

Uterine fluid from Group II contained six amino acids not detected in Group IV: unknown 1 (29/31) , taurine 5/31), phenylalanine (2/31), unknown 2 (9/31), histidine (8/31) and ornithine (7/31). The amino

acids in uterine fluid from Group I were similar to those in Group II, but did not contain taurine or ornithine; however, Group I did contain aspartic acid (1/29).

The presence of three to six additional amino acids in uterine fluid from Groups I, II, and III, when compared to Group IV (under more desirable management), represents an important change in uterine environment. This change in uterine fluid makeup could be responsible for the lower conception rate associated with the first estrus after withdrawal of progestins and with the higher rate of embryonic loss associated with heat stress.

The observed variations in amino acid and protein levels of uterine and oviductal fluids were not significantly different. However, it is noted that large differences would have had to exist for detection in the number of samples available in this study. Therefore small differences may exist that were not detected by this study.

The following conclusions appear to be valid based on data obtained in this study:

(1) There was variation among animals within and between treatments in the protein level and amino acid content of uterine and oviductal fluids. However, no statistically significant differences ($P < .05$) were detected;

(2) Uterine fluid collected after exsanguination consistently contained less protein, although not significant ($P < .05$), than fluid collected from live animals;

(3) Heat stress (Groups I and III) and/or heat stress and progestins (Group II) apparently cause, directly or indirectly, a change in the number of amino acids in the uterine and oviductal secretions of dairy heifers;

(4) The major amino acids in uterine fluid from dairy heifers maintained under a more desirable management were glutamic acid, threonine, alanine, glycine, arginine and serine.

These data from this study indicate that MGA and/or heat stress do effect the amino acid levels in secretions of the uterus and the oviduct. The specific mechanisms within the body that are being altered and how these mechanisms bring about changes in composition of uterine and oviductal fluid are not known.

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APPENDIX

APPENDIX TABLE 1. Amino acids detected most frequently in uterine fluid of dairy heifers on day 2 following non-synchronized estrus.

Animal number	Unknown #1	Glutamic acid	Threo-nine	Leucine[a]	Alanine	Clycine	Arginine	Lysine
	---mcg/ml---							
882	---	65.5	---	12.7	18.0	5.1	30.6	---
918	125.3	40.3	60.0	12.7	17.3	20.4	22.3	70.4
922	8.4	10.1	---	6.4	14.4	2.5	---	---
927	trace	10.1	---	2.1	14.4	---	---	---
5	62.6	55.4	93.0	---	15.8	33.1	44.5	---
6	41.8	85.7	76.6	---	15.8	17.8	50.1	30.7
7	45.9	25.2	---	---	5.8	7.6	---	---
8	62.6	75.6	38.3	46.7	37.4	50.9	239.9	---
9	16.7	20.2	24.6	---	8.6	6.4	5.6	---
10	87.7	65.5	43.8	29.7	5.8	---	---	---
11	66.8	45.4	46.5	---	10.1	20.4	44.5	---
12	54.3	105.8	128.6	---	25.9	86.5	61.3	184.3
16	trace	trace	---	---	---	---	---	---
Avg.[b]	47.6	46.7	64.0	18.4	15.8	25.1	62.4	95.1
Range	2.0-125.3	2.0-105.8	24.6-128.6	2.1-46.7	5.7-37.4	2.5-86.5	5.6-239.9	30.7-184.3

[a]Leucine and isoleucine reported as one value.

[b]Average based on number of samples containing the amino acid.

--- Amino acid was not detected.

Trace Averages as 2.0 mcg/ml.

APPENDIX TABLE 2. Amino acids detected less frequently in uterine fluid of dairy heifers on day 2 following non-synchronized estrus.

Animal number	Phenylalanine	Serine	Valine	Unknown #2	Histidine
			mcg/ml		
882	---	---	---	31.3	---
918	---	14.8	---	---	---
927	---	---	---	4.2	---
5	---	25.3	---	---	---
6	---	---	34.9	---	---
8	---	---	50.7	---	---
9	---	---	9.5	---	---
11	---	---	12.7	---	38.3
12	70.9	65.5	98.2	trace	---
Avg.[a]	70.9	35.2	41.2	12.5	38.3
Range		14.8-65.5	9.5-98.2	2.0-31.3	

[a]Average based on number of samples containing the amino acid.

Trace Averaged as 2.0 mcg/ml.

--- Amino acid was not detected.

APPENDIX TABLE 3. Total amount and number of amino acids in uterine fluid of non-synchronized and synchronized dairy heifers.

Animal number	Day 0 mcg/ml	Day 0 No.	Day 2 mcg/ml	Day 2 No.	Animal number	Day 0 mcg/ml	Day 0 No.	Day 2 mcg/ml	Day 2 No.
	----NON-SYNCHRONIZED----					------SYNCHRONIZED------			
5	347	10	330	7	5	840	12	637	9
6	264	8	323	7	6	125	10	390	11
7	225	9	85	4	-	--	--	--	--
8	326	12	602	8	9	524	7	568	8
9	210	9	92	7	9	321	7	857	8
10	363	8	233	5	10	1065	11	981	9
11	335	9	285	8	11	175	11	1910	13
12	723	11	883	11	12	950	10	79	6
14	586	10	--	--	14	160	7	--	--
16	484	11	4	2	--	--	--	--	--
17	628	9	--	--	17	--	--	729	8
18	129	6	--	--	18	131	6	--	--
20	615	9	--	--	20	62	7	100	9
882	94	8	163	6	1	262	9	814	10
918	--	--	384	9	2	1139	11	1627	12
922	35	5	42	5	3	--	--	881	10
927	419	8	33	5	920	556	8	16	6
					926	50	4	189	7
					K71	146	6	197	8
	GROUP III HOT					GROUP IV COOL			
5	466	7	--	--	1	114	7	--	--
6	362	6	--	--	2	76	6	95	6
9	174	6	--	--	4	228	7	--	--
10	374	8	--	--	7	378	6	885	5
11	681	9	388	7	8	237	8	106	6
14	213	7	975	7	12	176	9	266	7
16	583	6	--	--					
17	741	8	--	--					
18	815	8	1126	9					
20	751	9	580	9					

-- No data available.
No. Number of amino acids in the sample.

APPENDIX TABLE 4. Amino acids detected most frequently in uterine fluid of dairy heifers on day 0 following the first estrus after withdrawal of MGA.

Animal number	Unknown #1	Glutamic acid	Threo-nine	Leucine[a]	Alanine	Glycine	Arginine	Lysine
	mcg/ml							
920	192.1	90.7	114.9	---	17.3	30.5	22.3	30.7
926	37.6	---	---	4.2	2.9	5.1	---	---
933	trace	trace	trace	---	trace	trace	---	---
K71	16.7	45.4	35.6	---	7.2	7.6	33.4	---
1	---	20.2	31.5	---	13.0	25.4	61.3	23.0
2	346.6	trace	120.4	213.3	46.1	53.4	78.0	24.6
5	115.9	83.2	61.6	73.9	43.6	65.5	78.0	23.0
6	16.7	21.4	17.8	---	6.5	8.3	26.5	---
8	146.2	---	60.2	88.7	34.6	61.1	83.5	46.1
9	79.3	---	41.0	71.8	2.0	45.8	50.1	30.7
10	124.7	70.6	84.8	181.6	50.4	127.2	61.3	67.6
11	41.8	16.4	25.3	4.8	11.9	19.7	9.7	10.8
12	83.5	trace	87.6	141.5	51.8	104.3	211.6	---
14	50.1	20.2	32.8	---	11.5	20.4	---	9.2
18	---	20.2	43.8	---	5.8	trace	33.4	---
20	18.3	12.6	12.3	---	3.6	6.4	5.6	---
Avg.[b]	90.0	31.3	51.4	97.5	19.4	36.5	58.1	29.5
Range	2.0-346.6	12.6-90.7	2.0-120.4	4.2-213.3	2.0-51.8	2.0-127.2	5.6-211.6	9.2-67.6

[a]Leucine and isoleucine reported as one value.

[b]Average based on number of samples containing the amino acid.

Trace Averaged as 2.0 mcg/ml

--- Amino acid was not detected.

APPENDIX TABLE 5. Amino acids detected less frequently in uterine fluid of dairy heifers on day 0 following the first estrus after withdrawal of MGA.

Animal number	Taurine	Phenyl-alanine	Serine	Valine	Unknown #2	Histidine	Ornithine
	---mcg/ml---						
920	---	---	---	---	---	---	57.6
933	---	---	trace	---	---	---	---
1	57.6	---	15.8	14.3	---	---	---
2	---	---	---	63.4	158.7	32.8	---
5	74.9	---	---	47.5	134.7	38.3	---
6	---	---	5.3	7.9	7.3	6.8	---
8	---	---	---	---	---	49.8	---
10	---	17.7	---	---	221.3	---	57.6
11	---	---	7.4	11.9	---	---	15.6
12	---	---	---	82.4	108.6	76.6	---
14	---	---	---	15.8	---	---	---
18	---	---	---	25.3	---	---	---
20	---	---	---	2.2	---	---	---
Avg.[a]	66.2	17.7	7.6	30.1	126.1	40.8	43.6
Range	57.6-74.9		2.0-15.8	2.2-82.4	8.3-221.3	6.8-76.6	15.6-57.6

[a]Average based on number of samples containing the amino acids.

Trace Averaged as 2.0 mcg/ml.

--- Amino acid not detected.

APPENDIX TABLE 6. Amino acids detected most frequently in uterine fluid of dairy heifers on day 2 following the first estrus after withdrawal of MGA.

Animal number	Unknown #1	Glutamic acid	Threo-nine	Leucine[a]	Alanine	Glycine	Arginine	Lysine
	mcg/ml							
920	4.2	---	---	2.1	2.4	trace	trace	---
926	137.8	---	8.2	10.6	2.4	5.1	22.3	---
K71	62.6	25.2	2.7	---	13.0	15.3	27.8	27.6
1	167.0	136.1	114.9	---	21.6	53.4	139.2	21.5
2	346.6	110.9	191.5	67.6	72.0	190.8	105.8	110.6
3	225.5	80.6	98.5	---	8.6	40.7	44.5	55.3
5	225.5	---	71.1	109.8	20.2	61.1	27.8	43.0
6	75.2	37.8	71.1	---	20.9	33.1	13.9	32.3
8	104.4	---	10.9	65.5	27.4	63.6	217.2	24.6
9	193.0	110.9	158.7	---	34.6	101.8	155.9	30.7
10	114.9	70.6	98.5	152.1	51.8	56.0	128.1	---
11	213.0	110.9	120.4	264.0	141.1	165.4	239.4	12.3
12	29.2	15.1	---	8.5	2.9	10.2	---	---
17	125.3	141.1	104.0	---	28.8	96.7	89.1	67.6
20	23.0	12.6	16.4	---	5.8	8.9	11.1	15.4
Avg.[b]	128.0	77.4	82.1	85.0	30.2	60.3	87.4	40.1
Range	4.2-346.6	12.6-141.1	2.7-191.5	2.1-264.0	2.4-141.1	5.1-190.8	2.0-239.4	12.3-110.6

[a]Leucine and isoleucine are reported as one value.

[b]Average based on number of samples containing the amino acid.

Trace Averaged as 2.0 mcg/ml.

--- Amino acid was not detected.

APPENDIX TABLE 7. Amino acids detected less frequently in uterine fluid of dairy heifers on day 2 following the first estrus after withdrawal of MGA.

Animal number	Taurine	Phenyl-alanine	Serine	Valine	Unknown #2	Histidine	Arginine
	mcg/ml						
920	---	---	---	3.2	---	---	---
926	---	---	2.1	---	---	---	---
K71	---	---	23.2	---	---	---	---
1	---	---	48.6	---	---	49.2	62.4
2	---	88.6	---	215.4	45.9	---	81.6
3	207.4	---	33.8	---	---	---	86.4
5	---	---	---	60.2	---	---	19.2
6	23.0	---	27.5	42.8	12.5	---	---
8	---	---	---	53.9	---	---	---
9	---	---	71.8	---	---	---	---
10	---	---	---	---	254.7	54.7	---
11	103.7	---	147.8	253.4	83.5	54.7	---
12	---	---	---	12.8	---	---	---
17	---	---	---	76.0	---	---	---
20	---	---	2.1	4.8	---	---	---
Avg.[a]	111.3	88.6	44.6	80.3	99.2	52.8	62.4
Range	23.0-207.4		2.1-147.8	3.2-253.4	12.5-254.7	49.2-54.7	19.2-86.4

[a]Average based on number of samples containing the amino acid.

--- Amino acid was not detected.

APPENDIX TABLE 8. Amino acids and nitrogen compounds used to identify migrating bands on the electropherogram.

Amino Acids		Nitrogen compounds
Alanine	Methionine	Ammonia
Arginine	Ornithine	Urea
Aspartic acid	Phenylalanine	Thiourea
Citrulline	Proline	
Cysteine	Serine	
Glutamic acid	Taurine	
Glycine	Threonine	
Histidine	Tryptophan	
Leucine-isoleucine	Tyrosine	
Lysine	Valine	

APPENDIX TABLE 9. Randomized block 2X2 factorial analysis of variance for glutamic acid.

Source of variance	D.F.	Sum of squares	Mean squares	F
Total	27	38,670		
Animal	6	4,013	669	< 1
Treatment	3	5,059	1,686	1.0
Non-syn vs syn.	1	2,946	2,946	1.8
Day 0 vs Day 2	1	1,936	1,936	1.2
Interaction	1	178	178	< 1
Error	18	29,597	1,644	

APPENDIX TABLE 10. Amino acids detected most frequently in uterine fluid of dairy heifers on day 0 and day 2 following the second estrus after withdrawal of MGA (Group III).

Animal number	Unknown #1	Glutamic acid	Threo-nine	Leucine[a]	Serine	Alanine	Glycine	Arginine
	mcg/ml							
Day 0								
5	154.5	100.8	57.5	---	23.2	31.7	25.4	72.4
6	83.5	---	52.0	---	37.0	26.6	30.5	---
9	45.9	---	21.9	---	25.3	15.8	20.4	44.5
10	31.3	47.9	64.3	9.5	---	20.2	42.0	---
11	116.9	---	101.2	164.7	25.3	34.6	76.3	61.3
14	41.8	50.4	54.7	---	19.0	12.0	12.7	22.3
16	---	151.2	117.7	---	65.5	50.4	86.5	111.4
17	225.5	95.8	139.5	21.1	52.8	51.8	48.3	105.8
18	96.1	---	84.8	213.3	23.2	43.8	78.5	100.2
20	238.0	---	54.7	135.2	16.9	53.3	96.7	61.3
Avg.[b]	114.8	89.2	74.8	108.7	32.0	34.0	51.7	72.4
Range	41.8-238.0	47.9-151.2	21.9-139.5	9.5-213.3	16.9-65.5	12.0-53.3	12.7-96.7	22.3-111.4
Day 2								
11	108.1	---	---	59.1	19.0	33.1	38.2	66.8
14	238.0	---	93.0	---	38.0	51.8	91.6	128.1
18	158.6	126.0	125.9	---	52.8	43.2	53.4	161.5
20	54.3	65.5	109.4	21.1	---	31.7	48.3	55.7
Avg.	139.8	95.7	109.4	40.1	36.6	40.0	57.9	103.0
Range	54.3-238.0	65.0-126.0	93.0-125.9	21.1-59.1	19.0-52.8	31.7-51.8	38.2-91.6	55.7-161.5

[a]Leucine and isoleucine are reported as one value.

[b]Average based on number of samples containing the amino acid.

--- Amino acid not detected.

APPENDIX TABLE 11. Amino acids detected less frequently in uterine fluid of dairy heifers on day 0 and day 2 following the second estrus after withdrawal of MGA. (Group III)

Animal number	Taurine	Valine	Unknown #2	Lysine
	mcg/ml			
DAY 0				
6	132.5	---	---	---
10	121.0	38.0	---	---
11	---	79.2	---	21.5
18	---	---	175.4	---
20	---	85.5	---	9.2
Avg.[a]	126.7	67.6	175.4	15.3
DAY 2				
11	---	63.4	---	---
14	334.1	---	---	---
18	322.6	82.4	---	---
20	92.2	101.4	---	---
Avg.	249.6	82.4		

[a]Average based on number of samples containing the amino acid.

--- Amino acid was not detected.

VITA

William Carrol Loe was born June 29, 1931, at Route #5, Prescott, Hempstead County, Arkansas. He received his elementary and secondary education in Blevins Public School. Following graduation from high school in 1949 he enrolled at Southern State College (Magnolia A & M until the Fall of 1950), Magnolia, Arkansas. He transferred after two years to the University of Arkansas, Fayetteville, Arkansas and received his BSA in 1953. After serving in the U. S. Army from 1953 to 1955 he accepted a Vocational Agriculture teaching position at Lonoke High School, Lonoke, Arkansas, for the school year 1955-56. He accepted a similar position at Blevins High School for 1956-57.

After completing his second year of teaching he returned to the University of Arkansas in the Summer of 1957 to do graduate work in the Department of Dairy Science with emphasis on Production and Nutrition. He received his M.S. degree in January 1959 and accepted a position with Darragh Feed Company, Little Rock, Arkansas, as a Sales Representative. He returned to teaching Vocational Agriculture in 1960-61 at Blevins High School. He continued his education during the summer terms, receiving three National Science Foundation Institute grants. After 3 years he accepted a position as instructor in agriculture at Arkansas A and M College, Monticello, Arkansas. In 1966 he accepted a position as Assistant Professor of Agriculture at Southern

State College, Magnolia, Arkansas. He attended summer school at Colorado State University and Texas A and M University for one summer each. After 2 years at Southern State College, he was granted a two year leave of absence to attend Louisiana State University, Baton Rouge, Louisiana for advanced study in Physiology of Reproduction in the Department of Dairy Science. He is a candidate for the Doctor of Philosophy degree in August 1970.

He is married to the former Mildred E. Loofburrow of Yakima, Washington. They have three daughters, Janette Kay, Cathy Ann and Cynthia Gayle.

EXAMINATION AND THESIS REPORT

Candidate: William Carrol Loe

Major Field: Dairy Science (Physiology of Reproduction)

Title of Thesis: Protein and Amino Acid Content of Uterine and Oviductal Fluid of Dairy Heifers

Approved:

T. E. Patrick
Major Professor and Chairman

Max Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

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Date of Examination:

July 10, 1970